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Report Title

Hybrid Control Models and Tools for Biological Regulatory Networks: Final Report

ABSTRACT

This report describes the research completed under Research Agreement DAAD19-03-1-0373, awarded to Stanford University as part of DARPA's BioComputation Program. The overall goal of this research is to design mathematical models and analysis techniques, based on control theory and hybrid systems, to help understand intra- and inter-cellular biological regulatory networks. One of the products of this research was integrated into the BioSPICE tool, developed by SRI and providing a common framework for the different methods developed as part of the BioComputation program.
The research performed under this agreement produced: (i) a procedure, based on deterministic and stochastic hybrid system reachability tools, for identifying parameters of different biological systems; (ii) a technique for identifying parameters for continuous state models of protein regulatory networks; (iii) new insights, provided by these tools, into the operation of the mechanisms behind Planar Cell Polarity in Drosophila wings, and into the survival analysis of Bacillus subtilis.

List of papers submitted or published that acknowledge ARO support during this reporting period. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Amonlirdviman, K., Khare, N.A., Tree, D.R.P., Chen, W.-S., Axelrod, J.D. and Tomlin, C.J. (2005) Mathematical modeling of planar cell polarity to understand domineering nonautonomy. Science 307, 423-426.

C. J. Tomlin and J. D. Axelrod, Understanding biology by reverse engineering the control, Proceedings of the National Academy of Sciences (PNAS) 102(12), pp. 4219-4220, March 22, 2005.

R. Ghosh and C. J. Tomlin,
Symbolic reachable set computation of piecewise affine hybrid automata and its application to
biological modeling: Delta-Notch protein signaling,
IEE Systems Biology, Volume 1, Number 1, pp. 170--183, June 2004.

Number of Papers published in peer-reviewed journals: 3.00

(b) Papers published in non-peer-reviewed journals or in conference proceedings (N/A for none)

Number of Papers published in non peer-reviewed journals: 0.00

(c) Presentations

Tomlin:

Plenary Speaker, International Symposium on Advanced Control of Chemical Processes, Gramado, Brazil, April 2006.

Invited Speaker, International Conference on Systems Biology, October 2005.

Plenary Speaker, NIH Conference on Molecular Systems Biology, Tahoe City, August 2004.

Plenary Speaker, American Control Conference, Boston, June 2004.

First of the ``Talks in English'', Stanford University Bio-X Program, February 2004.

``An Engineering Viewpoint of Signaling in Protein Networks'',
National Academies' Keck Foundation Initiative for Interdisciplinary Science, Washington DC, May 2003.

Amonlirdviman:

"Mathematical Modeling to Understand Planar Cell Polarity", poster talk given at the International Conference on Systems Biology, Heidelberg, October 2004. (Winner First Prize, Best Poster Talk)

Number of Presentations: 7.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts): 0

Peer-Reviewed Conference Proceeding publications (other than abstracts):

R. Raffard, K. Amonlirdviman, J. D. Axelrod, and C. J. Tomlin,
Automatic parameter identification via the adjoint method, with application to understanding planar cell polarity, In the Proceedings of the IEEE Conference on Decision and Control, San Diego, December 2006.

A. Abate, A. Tiwari,
"Box Invariance of hybrid and switched systems", in the Proceedings of the 2nd IFAC Conference on Analysis and Design of Hybrid Systems, Alghero, IT, 2006.

R. Ghosh and C. J. Tomlin, A Query-Based Technique for Interpreting Reachable Sets for Hybrid Automaton Models of Protein Feedback Signaling, In the Proceedings of the AACC American Control Conference, Portland, June 2005.

R. Ghosh and C. J. Tomlin, An Algorithm for Reachability Computations on Hybrid Automata Models of Protein Signaling Networks, Proceedings of the 43rd IEEE Conference on Decision and Control,
Atlantis, Bahamas, pp. 2256--2261, December 2004.

J. Hu, W.-C. Wu, and S. Sastry, "Modeling subtilin production in Bacillus subtilis using stochastic hybrid systems," Hybrid Systems: Computation and Control, 7th Int. Workshop (HSCC 2004), Philadelphia, PA, R. Alur, and G. J. Pappas, eds., vol. 2993 of Lecture Notes in Computer Science, Springer-Verlag, pp. 417-431, 2004.

R. Ghosh, A. Tiwari, and C. J. Tomlin, Automated Symbolic Reachability Analysis with application to Delta-Notch Signaling}, Springer-Verlag Lecture Notes in Computer Science (LNCS 2623),
Maler and Pnueli (Eds.), pp. 233-248, March 2003.

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts): 6

(d) Manuscripts

Ma, D., Amonlirdviman, K., Tomlin, C. and Axelrod, J.D. (submitted) Cell packing influences planar cell polarity signaling.

A. Abate, A. Tiwari, S. Sastry,
"The concept of Box Invariance for biologically-inspired dynamical systems", UCBerkeley, EECS Dept. Technical Report.

Number of Manuscripts: 2.00

Number of Inventions:

Graduate Students

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Ronojoy Ghosh	0.50	No
Keith Amonlirdviman	0.10	No
Dali Ma	0.50	No
Jianghai Hu	0.50	No
Wei-Chung Wu	0.50	No
Alessandro Abate	0.25	No
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Total Number:	**6**	

Names of Post Doctorates

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Narmada Khare	0.50	No
FTE Equivalent:	**1.00**	
Total Number:	**2**	

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NAME	PERCENT_SUPPORTED	National Academy Member
Claire Tomlin	0.10	No
Jeffrey Axelrod	0.10	No
S. Shankar Sastry	0.03	Yes
FTE Equivalent:	**0.23**	
Total Number:	**3**	

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NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Personnel receiving masters degrees

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Ronojoy Ghosh	No
Keith Amonlirdviman	No
Dali Ma	No
Total Number:	**3**

Names of personnel receiving PHDs

NAME	
Ronojoy Ghosh	No
Keith Amonlirdviman	No
Dali Ma	No
Total Number:	**3**

Names of other research staff

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FTE Equivalent:	
Total Number:	

Sub Contractors (DD882)

Inventions (DD882)

Hybrid Control Models and Tools for Biological Regulatory Networks

C. J. Tomlin, J. D. Axelrod, and S. Shankar Sastry

Statement of the Problems Studied

The overall goal of this research is to design mathematical models and analysis techniques, based on control theory and hybrid systems, to help understand intra- and inter-cellular biological regulatory networks. The research performed under this agreement produced: (i) a procedure, based on deterministic and stochastic hybrid system reachability tools, for identifying parameters of different biological systems; (ii) a technique for identifying parameters for continuous state models of protein regulatory networks; (iii) new insights, provided by these tools, into the operation of the mechanisms behind Planar Cell Polarity in Drosophila wings.

The procedure for hybrid system reachable set calculation was integrated into the BioSPICE tool, developed by SRI and providing a common framework for the different methods developed as part of DARPA's BioComputation program.

Summary of the Most Important Results

(i) Hybrid systems models for parameter identification in biological systems

Hybrid automata are an eminently suitable modeling framework for biological protein regulatory networks, as the protein concentration dynamics inside each biological cell are modeled using linear differential equations; inputs activate or deactivate these continuous dynamics through discrete switches, which themselves are controlled by protein concentrations reaching given thresholds. This research proposed an iterative refinement algorithm for computing discrete abstractions of a class of deterministic hybrid automata with piecewise affine continuous dynamics and forced discrete transitions, defined completely in terms of symbolic variables and parameters. Furthermore, these discrete abstractions are utilized to compute symbolic parametric backward reachable sets from the equilibria of the hybrid automata, that are guaranteed to be exact or conservative under approximations. The algorithm was implemented using MATLAB and QEPCAD, to compute reachable sets for the biologically observed equilibria of the multiple cell Delta Notch protein signaling automaton with symbolic parameters. The results were analyzed to show that novel, non-intuitive, and biologically interesting properties can be deduced from the reachability computation, thus demonstrating the utility of the algorithm [3, 7, 9].

In some cases, a stochastic model is appropriate to model the biological system in question: we modeled the genetic network regulating the biosynthesis of subtilin in Bacillus subtilis as a stochastic hybrid system. The continuous state of the hybrid system is the concentration of subtilin and various regulating proteins, whose productions are controlled by switches in the genetic network that are in turn modeled as Markov chains. This model allows one to reinterpret the survival analysis for the single *B. subtilis* cell and study it as a probabilistic, decentralized safety specification problem over a short

time horizon. Using recently developed techniques for probabilistic verification in a stochastic hybrid systems setting, we reinterpret the above probabilistic safety problem as a (stochastic) optimal control one, where the controls are (possibly randomized) functions of the state-space that encode the switches in the network. Finally, the solution of this short-time-horizon, stochastic and decentralized optimal control problem yields the structure of the switching behaviors under study. Matching these outcomes with the data in the literature allows concluding that the corresponding mechanisms in the subtilin production network function with a degree of optimality, according to certain survival criteria [8].

We introduce a special notion of Invariance Set for certain classes of dynamical systems: the concept has been inspired by our experience with models drawn from Biology. We claim that Box Invariance, that is, the existence of "boxed" invariant regions, is a characteristic of many biologically-inspired dynamical models, especially those derived from stoichiometric reactions. Moreover, box invariance is quite useful for the verification of safety properties of such systems. This paper presents effective characterization of this notion for linear and affine systems, the study of the dynamical properties it subsumes, computational aspects of checking for box invariance, and a comparison with related concepts in the literature. The concept is illustrated using two models from biology [5, 10].

(ii) A technique for identifying parameters for continuous state models of protein regulatory networks.

A key focus of systems biology has been the development of models, at the appropriate level of abstraction, to help understand different biological processes. This development usually proceeds in iterative fashion, in which the structure of the model is chosen to represent certain hypotheses about how the system operates and parameters for this structured model are chosen. Often, the first experiment is to ask if a robust set of parameters exists so that the model reproduces all or most of the observed biological data. The model is tested against this actual data and for its predictive capabilities. As new data and/or new understanding arise, the structure of the model may be altered, and new parameters selected. In protein regulatory networks, the number of states to model is typically large and depends on the number of proteins of interest, the parameter spaces are large, and the most appropriate models are nonlinear functions of the states. Thus it is becoming increasingly important to develop fast, efficient, scalable methods for large scale parameter identification. This research identifies methods for performing automatic parameter identification on differential equation based models of biological systems. The first method is based on the Nelder-Mead Simplex Method [1], the second is an Adjoint-based algorithm [4]. In both cases, the algorithm solves an optimization problem, in which the cost reflects the deviation between the observed data and the output of the parameterized mathematical model, and the constraints reflect the governing parameterized equations themselves.

(iii) New insights into Planar Cell Polarity.

The algorithms described in (ii) above have led to two major advances in our understanding of the regulatory network that controls planar cell polarity in the fly wing epithelium. The first was to assess the feasibility of a proposed biochemical network model to explain complex, tissue level patterns seen in a variety of mutant conditions. Based on genetic experiments performed in the lab, the model was constructed, validated, and used to demonstrate not only the feasibility of the proposed network model, but its robustness. Furthermore, the model led to predictions about how to explain an unusual class of mutant allele of *frizzled*, one of the genes involved. Experiments were done in the lab to demonstrate that the predictions were correct. This work was published in [1].

The second major advance was to understand the origins of a variable mutant phenotype. We found that disruption of polarity within clones of cells mutant for one of the signaling components, *fat*, is variable. Based on observations, we proposed that a variable cell geometry could explain the variable phenotype. Biological and computational experiments were performed that quantified the correlation between cell geometry and polarity disruption, and biological experiments demonstrated a cause and effect relationship between cell geometry and polarity. Finally, the mathematical model described in Amonlirdviman et al. was modified to run on cell geometries that are extracted from images of real wings, and it was shown that our proposed signaling network, when confronted with real cell geometry, explains most if not all of the variability in *fat* clone phenotypes. This work is described in [2].

Finally, in recent work, we used the model in [2] to address a question concerning the polarity with which Fat communicates with Frizzled. While the simulations in Ma et al. provided reasonable approximations, we realized that by simulating clone boundary effects, we could deduce the polarity of the Fat-Frizzled linkage, and indeed the simulations demonstrate that linkage in one orientation considerably improves the result, while linkage in the other orientation worsens it. The simulations therefore favor one linkage. This conclusion will be tested against other biological results that should depend on this linkage.

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2. Ma, D., Amonlirdviman, K., Tomlin, C. and Axelrod, J.D. (submitted) Cell packing influences planar cell polarity signaling, 2006.

3. R. Ghosh and C. J. Tomlin, Symbolic reachable set computation of piecewise affine hybrid automata and its application to biological modeling: Delta-Notch protein signaling, IEE Systems Biology, Volume 1, Number 1, pp. 170--183, June 2004.

4. R. Raffard, K. Amonlirdviman, J. D. Axelrod, and C. J. Tomlin, Automatic parameter identification via the adjoint method, with application to understanding planar cell polarity, In the Proceedings of the IEEE Conference on Decision and Control, San Diego, December 2006.

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6. R. Ghosh and C. J. Tomlin, A Query-Based Technique for Interpreting Reachable Sets for Hybrid Automaton Models of Protein Feedback Signaling, In the Proceedings of the AACC American Control Conference, Portland, June 2005.

7. R. Ghosh and C. J. Tomlin, An Algorithm for Reachability Computations on Hybrid Automata Models of Protein Signaling Networks, Proceedings of the 43rd IEEE Conference on Decision and Control, Atlantis, Bahamas, pp. 2256--2261, December 2004.

8. J. Hu, W.-C. Wu, and S. Sastry, "Modeling subtilin production in Bacillus subtilis using stochastic hybrid systems," Hybrid Systems: Computation and Control, 7th Int. Workshop (HSCC 2004), Philadelphia, PA, R. Alur, and G. J. Pappas, eds., vol. 2993 of Lecture Notes in Computer Science, Springer-Verlag, pp. 417-431, 2004.

9. R. Ghosh, A. Tiwari, and C. J. Tomlin, Automated Symbolic Reachability Analysis with application to Delta-Notch Signaling, Springer-Verlag Lecture Notes in Computer Science (LNCS 2623), Maler and Pnueli (Eds.), pp. 233-248, March 2003.

10. A. Abate, A. Tiwari, S. Sastry, "The concept of Box Invariance for biologically-inspired dynamical systems", UCBerkeley, EECS Dept. Technical Report.